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Laboratory rearing of western corn rootworms and hybridization of the western corn rootworm (Diabrotica Virgifera) and northern corn rootworm (D. Longicornis) (Coleoptera: Chrysomelidae)

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LABORATORY REARING OF WESTERN CORN ROOTWORMS AND HYBRIDIZATION OF THE WESTERN CORN ROOTWORM (*DIABROTICA VIRGIFERA*) AND NORTHERN CORN ROOTWORM (*D. LONGICORNIS*) (COLEOPTERA: CHRYSOMELIDAE).

BY

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LABORATORY REARING OF WESTERN CORN ROOTWORMS AND HYBRIDIZATION OF THE WESTERN CORN ROOTWORM (*DIABROTICA VIRGIFERA*) AND NORTHERN CORN ROOTWORM (*D. LONGICORNIS*) (COLEOPTERA: CHRYSOMELIDAE).

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser — Date: January 15, 1965

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GENERAL INTRODUCTION

The corn rootworms are beetles of the family Chrysomelidae, genus Diabrotica whose larvae feed on the roots of corn. The northern corn rootworm, Diabrotica longicornis (Say), has been known as an occasional serious pest of corn for many years. Large populations of the western corn rootworm, Diabrotica virgifera LeConte, recently developed in Nebraska and surrounding states, thus increasing the economic importance of these insects.

Widespread soil insecticide treatments for both species were adopted after 1951 (Lilly, 1956) and have become widely accepted. In South Dakota the number of acres treated for rootworm control rose from 122,715 in 1959 (U. S. Dep. Agr., 1960) to 750,000 in 1963 according to unpublished estimates by county agents. However, the recommended methods of control have recently become ineffective in some areas; as a result crop losses due to these pests have risen sharply. Considering their economic importance, little is recorded about the biology of rootworms. Perhaps this is because they are soil insects. This study was undertaken to provide some of the basic information necessary to research on better methods of control.

The rootworms were collected early in the history of American entomology. They were not recognized as pests until intensified and specialized agriculture was established. John L. LeConte (1868) first described D. virgifera from specimens collected near Fort Wallace, in Wallace County, Kansas. The western corn rootworm (WCR)

was recognized as a pest of corn in Colorado by C. P. Gillette (1912) who reported that the larvae were feeding on and damaging corn roots. He recommended crop rotation as a simple remedy.

D. longicornis was described by Thomas Say (1824) following his 1823 collections in the Arkansas Territory. French in the 11th Illinois state entomologist report (1882) credited Charles V. Riley with the first discovery of the northern corn rootworm as a pest of corn in Missouri in 1879. Thomas and French reported in the 10th and 11th Illinois state entomologist reports (1881, 1882) severe damage to corn by northern corn rootworms (NCR) in Illinois during 1880 and 1881 and suggested crop rotation as a means of control. The NCR was common in Illinois but had not been found feeding on corn prior to that time.

WCR adults are yellow on the venter of the head, abdomen, tibia, and the dorsum of the thorax. The dorsum of the abdomen is yellow with dark brown transverse plates. The dorsum of the head and the legs, with the exception mentioned above, are dark brown. The venter of the metathorax is light brown, and the elytra are nearly a solid dark brown or yellow with dark brown stripes. The yellow parts of the body may vary from bright yellow to bright green, gray, or tan. The NCR have a nearly uniform color and are slightly smaller than the WCR. They may be bright green, pale green, or a light tan. Some NCR have been found with a gray or dark colored elytron, others have dark lines around the margins of the elytra, and some have narrow stripes on the elytra. However there usually is no difficulty in determining

the species, and all the variously colored beetles may be collected from the same area.

Members of the two species frequently have been seen in mating position in the field. The possibility of interspecific hybridization has been considered because of the attempted matings of the two species and the wide variations in color patterns of the two beetles found in the field.

The life histories of the two insects appear to be similar. In South Dakota eggs are laid in corn fields from mid-August until killing frost. These eggs overwinter and are assumed to hatch the following May or June. The larvae eat on and burrow in corn roots with the entire root system being destroyed if a sufficiently heavy infestation occurs. Such weakened plants may then lodge following strong wind or rain. If adequate moisture is present the root system regenerates and the plant recovers from the initial larval attack. Pupation takes place in pupal chambers formed in the soil by the mature larvae with adult emergence beginning in July and continuing for several weeks. The beetles feed on the corn plant above ground, particularly the silks and pollen. When the silks are eaten back to the tip of the ear before pollination is complete only part of the kernels on the ear develop. While both the adults and larvae are damaging to corn, the larval state is considered the most injurious.

The WCR developed large damaging populations in central Nebraska by 1960. These spread to central Iowa, South Dakota,

Minnesota, and Missouri by 1964. The reasons for this movement are not adequately understood. The environment is especially favorable for rootworms in the Platte River valley (Weekman, 1961). Whether the rootworms migrated from this area or not is a matter of speculation. Some workers feel that they migrate into new areas and rapidly expand their numbers; others feel that the rapid increase in population is the result of a build-up of a low resident population that accompanied the practice of planting continuous corn.

The development of resistance by the WCR to the chlorinated hydrocarbons, aldrin and heptachlor, has also followed the spread of economic populations (Weekman, 1961) to other states. This has increased populations in areas where resistance has developed. Two possible causes for the expanding populations then are good growing conditions and insecticide resistance. There are however too many unexplained observations to use this as a complete hypothesis.

With the part of this background information that was available in 1962, studies were initiated to determine if interspecific hybridization could occur. The results are reported here in two sections. The first part was an exploratory investigation into the methods of handling the insects while the second dealt with the hybridization experiment.

PART I

TESTS OF DIETS, CAGES, AND POPULATION DENSITIES

Introduction

Insects which are reared in the laboratory are placed in a completely new and artificial environment. Therefore, the development of methods which minimally affect the insects and yet allow for efficient handling is prerequisite for further studies. Food which is readily available, easy to handle, and does not adversely affect fecundity is a primary consideration.

Published reports of food selected by rootworm beetles in the field are numerous. The first WCR described were collected by LeConte (1868) from wild gourd. They have been reported (Brisley, 1926) as feeding on the petals and pollen of wild gourd, Cucurbita foetidissima H. B. K., and on the leaves, stems, petals, and pollen of watermelons, squash, lettuce, cucumbers, muskmelons, and beans. In the 10th, 11th, and 18th Illinois state entomologist reports (1881, 1882, and 1891-1892) Thomas, French, and Forbes reported the NCR as feeding on pollen from the flowers of various Compositae, ragweed, thistles, squash, red clover, and the vines of cucumbers and squash, and other plants in bloom in the corn field.

Burkholder (1956) tested the effect of different diets on fecundity. He found the WCR laid more eggs when fed on squash flowers, corn pollen, and silk than on any other diet; however, they laid next to the lowest number of eggs when fed on corn silk alone.

Corn silks were retested in this experiment because this is a primary adult food in the field.

Diet and caging have frequently been reported to affect the longevity of insects. For example, Atwal (1955) found that when larvae of the diamond-back moth, Plutella maculipennis (Curtis), were fed on immature leaves of cabbage rather than mature leaves, the resulting moths lived longer. Davis (1945) observed that the longevity of Trogoderma versicolor Creutz. females was decreased with increasing densities of the cage population. For these reasons the effects of diet, cage style, and population density on the longevity of corn rootworm beetles were studied in this experiment.

Materials

All beetles were kept in one of the two cage styles illustrated in Fig. 1 and 2. The larger cage, 25 $in.^3$, was made of 2-inch cellulose butyrate tubing (Fig. 1). Ventilation holes in the cage were covered with 20-mesh plastic screen. Access to the cage was through a small hole in the side of the tube which was plugged with a cork. The top of the cage was fitted with a 2-inch cork into which was inserted a small glass vial to hold food material. The beetles oviposited through a 2-inch disc of 16-mesh copper screen glued to the bottom of the tubing. The oviposition site was a pad of cellucotton, commercially prepared creped cellulose, in the bottom of a paraffin coated 2-inch souffle cup placed directly beneath the copper screen.

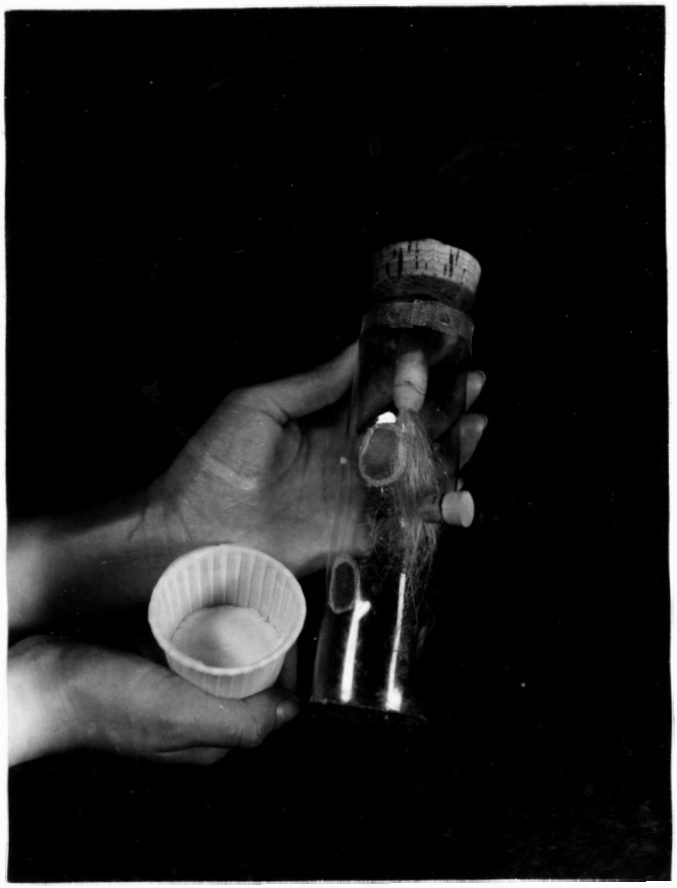

Fig. 1. Large cage (25 in.^{3}) with green corn silk.

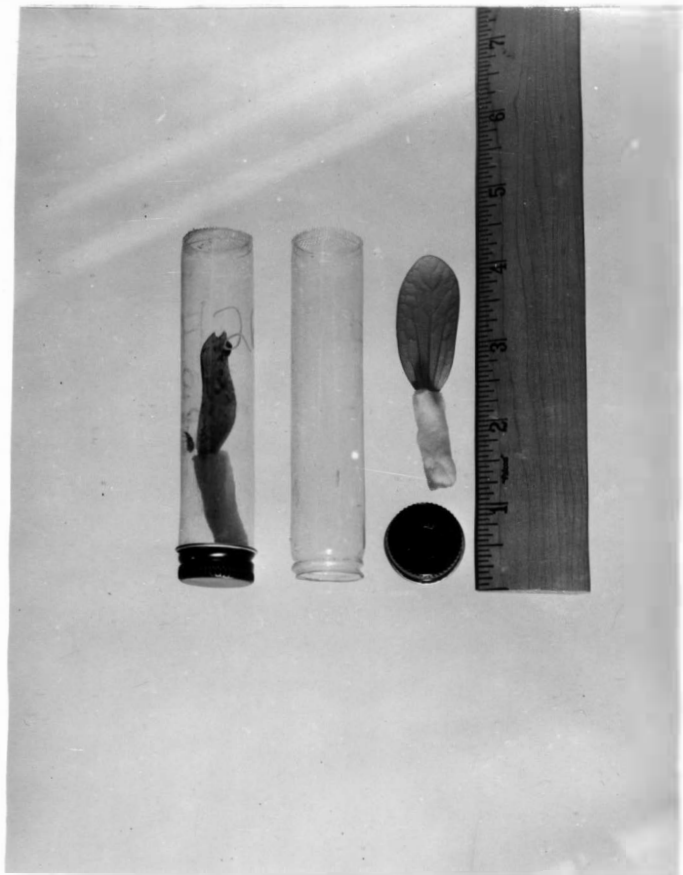

Fig. 2. Small cage (2.5 in.^{3}) with a cucurbit cotyledon.

The smaller cage, 2.5 in.^{3}, was made from a polystyrene screw cap vial, 7/8 in. by 4 in. (Fig. 2). The bottom of the vial was removed and a piece of 20-mesh plastic screen was glued on the open end to allow ventilation. The metal cap served as a base on which to stand the cage. Entry was gained through the mouth of the vial. A small piece of moist cotton placed inside the cap served as a site for oviposition.

The five kinds of food used were: Early Prolific Straightneck and Black Zucchini squash cotyledons, green corn silk, honey bee pollen substitute[1/] mixed with a 1:1 solution of honey and distilled water, artificial diet (Table 5) devised for the European corn borer, Ostrinia nubilalis (Hübner) by Becton, George and Brindley (1962), and the artificial diet for European corn borers modified by using a water extract of green silk and non-nutritive cellulose in place of distilled water and the leaf factor. Food was placed in the cages by several methods. In the small cages the three soft diets, pollen substitute mixture, the artificial diet for European corn borers, and the modified artificial diet, were pressed onto one side of the ventilation screen. The diets were kept moist by sprinkling with water several times daily. Green corn silk was placed in a loose ball in the bottom of the cage, and the petioles of the cucurbit cotyledons were wrapped in moist cotton and placed cotton side down

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[1/] 1 part brewer's yeast, 6 parts expeller processed soybean flour.

in the cage. This moist cotton served as the site for oviposition and as a source of moisture for the cotyledon.

In the large cages, the three soft diets were placed on metal troughs inserted into the cork which closed the access port of the cage. The diets could be replenished by simply removing the cork in the side of the cage. Corn silk and cucurbit cotyledons were placed in water-filled vials and held in position with cotton.

The cages were supported by wooden racks (Fig. 3) designed to hold 16 of the large cages. The small cages were easily leaned against the back of the rack. The cages were held at room temperature and artificial light.

The following information was recorded: date of collection of the beetles, date of oviposition and number of eggs laid, date of death and sex of the dead beetles, date of refrigeration and incubation of the eggs, and dates of hatching.

Procedures

Adult WCR beetles were field collected 14 and 22 August 1962 from Canton, South Dakota. The NCR were collected daily at Brookings, South Dakota from a field cage containing 21 corn plants. The sex of the beetles was determined prior to caging. Beetles were placed in cages according to the experimental design shown in Table 1. All tests were conducted with both NCR and WCR. The five kinds of food were tested in four cages at three different population densities,

Fig. 3. Wooden rack holding large cages under VHO fluorescent lamps.

except for omissions due to experimental error. This resulted in 20 cages each of WCR and NCR.

Table 1. Experimental design used in caging WCR and NCR beetles.

Population densities		25 in.^{3} cage[a]	2.5 in.^{3} cage
(number of beetles)		(number of beetles)	
Low	1/2.5 in.^{3}	10	--
Medium	1/1 in.^{3}	25	4
High	4/1 in.^{3}	--	10

[a] Equal numbers of males and females were placed in the cages except for one additional male where odd numbers are used.

Food was replaced when the amount was insufficient to support the cage population or when the soft diets appeared dehydrated. The cellucotton oviposition sites or egg papers were checked for eggs at this time. The egg papers were moistened twice daily or as often as needed.

When the egg papers were removed they were moistened, placed in petri dishes or stender dishes, and left at room temperature approximately two weeks to allow for any embryonic development that might normally occur prior to overwintering of the eggs. Then the eggs were refrigerated at 1.5°C in order to simulate winter temperatures diapausing eggs encounter in the field. During refrigeration most of the egg papers dehydrated and were remoistened. The dishes containing the eggs were then sealed in polyethylene bags; however this

did not prevent the small egg papers from dehydrating. Refrigeration was discontinued after 194 to 294 days and the eggs were incubated at 30°C.

Results and Conclusions

The effect of food, caging, and population density on mortality and fecundity of WCR and NCR adults are considered below. All three factors affected every cage of insects since each cage represented a unique combination of food, population density, and cage style. As a result the data given are combinations of the influences of several factors. For example, when longevity of all beetles held at the medium population density is considered, the calculated average represents the longevity of all beetles held at medium density regardless of diet and cage style.

There is a certain amount of missing data which must be considered when interpreting the graphs presented in this section. Ideally, each of five different diets would have been tested in two cage styles at three population densities, but a lack of beetles prevented completion of the experimental design. In addition, mortality records from some cages were incomplete. Table 2 shows the completed experimental design and a summary of the records of mortality.

Longevity of the WCR beetles was more affected by diet than by cage style or population density. Mortality rates on the five diets are shown in Fig. 4. The sigmoid curves on the graph

Table 2. Cages used in experimental procedures.

Diet	Adult population density in 25 in.^{3} cages: 1/2.5 in.^{3}	25 in.^{3} cages: 1/1 in.^{3}	and 2.5 in.^{3} cages: 1/1 in.^{3}	2.5 in.^{3} cages: 4/1 in.^{3}
Cucurbit cotyledons	X[a]	X[b]	X[b]	X
Green corn silk	X[c]	X	No cage	X[d]
Honey bee pollen substitute mixture	No cage	X[b]	X	X
Artificial diet for European corn borers	X	X	X	X
Modified artificial diet for European corn borers	X	X[b]	X	X

[a] X indicates cage present with adequate records of beetle mortality.
[b] Cage present with insufficient records of beetle mortality.
[c] Cage present with no record of beetle mortality.
[d] Two replications.

represent the mortality rates of beetles fed on the artificial diet for European corn borers (ECB) and the same diet modified with a silk extract and non-nutritive cellulose. These two diets are the only diets which were tested in all or nearly all of the cages (Table 2). Honey bee pollen substitute mixture supported beetles the longest so the top of the rate-of-mortality curve for that diet extends to the right farther than for any other diet. Since information on large cages of insects fed on honey bee pollen substitute mixture was missing, mortality curves pertaining to large cages or the low population density will never be extended as far to the right as those curves which include beetles fed on honey bee pollen substitute mixture. When the limited information available for mortality of beetles fed on honey bee pollen substitute mixture in the large cage at medium density is added to Fig. 4, it appears that if these data had been complete the top of the original curve for honey bee pollen substitute mixture may not have been extended so far to the right. Therefore, the validity of the upper portion of the curve is questionable. The effect of this diet will also be seen in the comparisons made in Fig. 5 and 6.

The rapid rate of mortality on the modified ECB diet indicates that this was a completely unsatisfactory food. The ECB diet and cucurbit cotyledons would be satisfactory for short term feeding but were not adequate to maintain beetles through their possible adult life span. Green corn silk and the honey bee pollen substitute mixture supported beetles adequately although there was a rapid rate

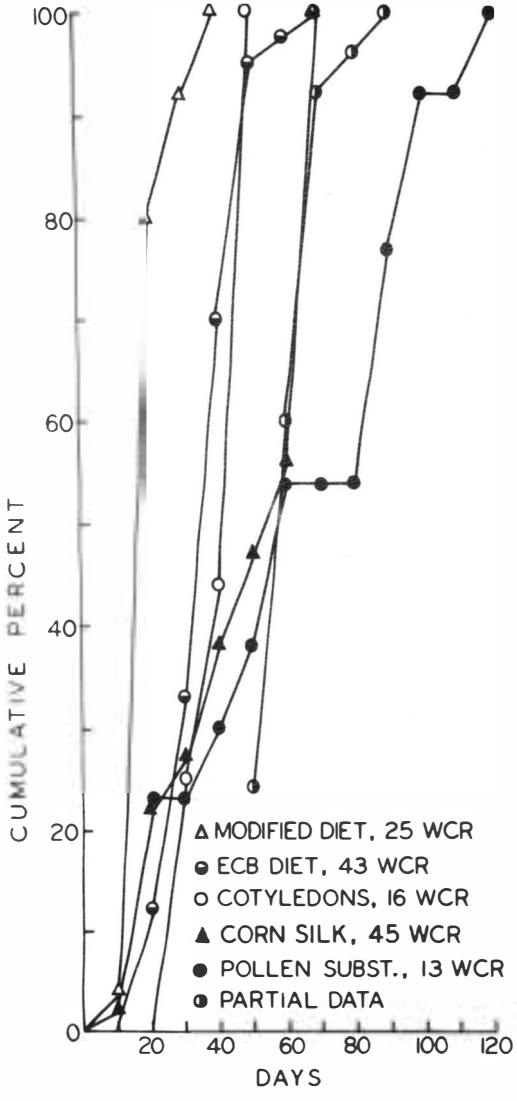

FIG. 4 MORTALITY ON DIETS.

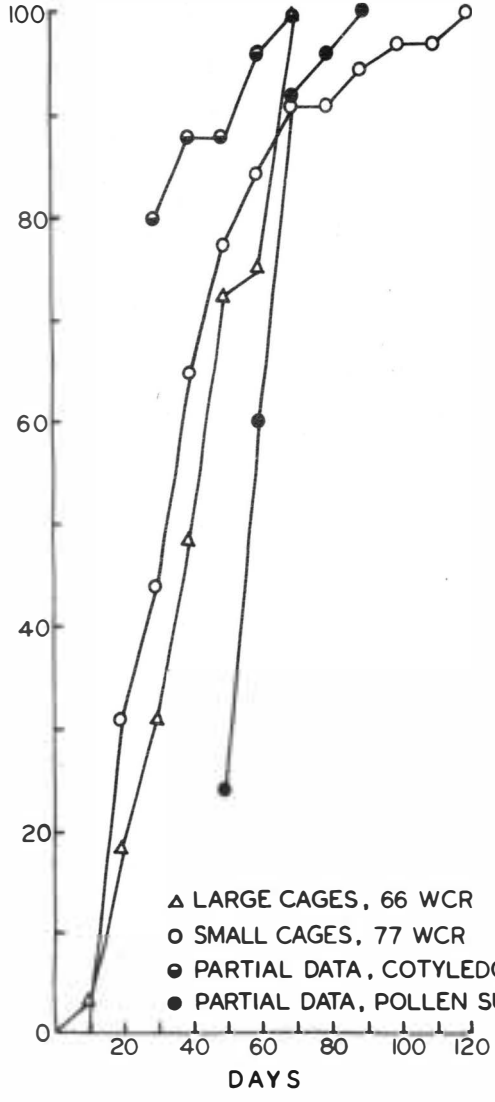

FIG. 5 MORTALITY IN CAGES.

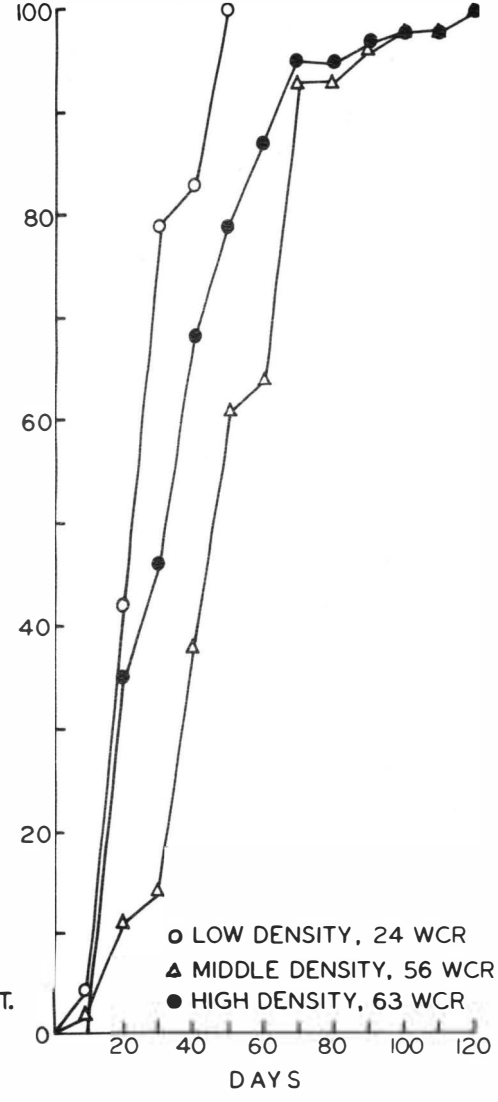

FIG. 6 MORTALITY AT DENSITIES.

of mortality during the early part of the adult stage. Honey bee pollen substitute mixture definitely appears to be the most complete diet tested with beetles surviving up to 120 days. While beetles fed on honey bee pollen substitute mixture lived longer than on the other diets tested, it does not appear to be the most adequate diet when factors besides longevity are considered.

Fig. 5 compares the longevity of beetles when held in the two different cages. Nearly equal numbers of beetles were tested in the two cage styles. All diets were represented in the small cages; however, data from the beetles at both densities in large cages fed on the honey bee pollen substitute mixture were incomplete or missing (Table 2). Cucurbit cotyledons, the modified diet for ECB and green corn silk were tested in only one of the two large cages. The two portions of incomplete data from beetles held in the large cages at medium density and fed on cucurbit cotyledons and honey bee pollen substitute mixture are included in Fig. 5. Perhaps if these partial data had been included the top one-quarter of the large-cage curve, except for the last four 10-day periods, would have been brought more in line with the mortality curve for small cages. However, the two curves are quite similar indicating that caging, within the limits tested here, did not greatly affect longevity.

The effect of the three population densities on rate of mortality is shown in Fig. 6. The effect of honey bee pollen substitute mixture on the mortality curves representing the medium and

high densities, 56 and 63 beetles respectively, is seen again as extending these two curves far to the right. The low density curve with 24 individuals does not include tests of the two diets, honey bee pollen substitute mixture and green corn silk, which sustained beetles the longest. The curve would probably have shifted to the right if they had been included. All diets were tested at the high population density level. The curve representing the middle population density included only five of a possible ten cages (Table 2) and is so incomplete that it is inaccurate to compare it with the other two densities. However, by comparing the rate-of-mortality curves for the low and high densities it appears that the population densities tested here had no marked affect on longevity of the beetles. It would have been desirable to compare the mortality rates of the two groups of beetles held at the middle density because any difference shown would have been the effect of cage style. This comparison was not made because of the great difference in numbers of beetles held in the two cage styles and the inadequate mortality data (Table 2).

Table 3 summarizes the oviposition records from the WCR cages. Responses of the beetles were inconsistent; however, some trends can be seen. Cage style does not consistently show an effect on fecundity. The average number of eggs laid per female is slightly greater in the small cages, but there is sufficient variation so it must be concluded within the cage styles tested here there was no difference in their effects on fecundity.

Table 3. Summary of the effects of diet, cage style, and population density on the fecundity of WCR expressed as average number of eggs produced per female.

Diet	Average number of eggs produced per female				
	Avg by diet	Adult population density in 25 in.^{3} cages		and	2.5 in.^{3} cages
		1/2.5 in.^{3}	1/1 in.^{3}		1/1 in.^{3}	4/1 in.^{3}
Cucurbit cotyledons	28	10	51		13	14
Green corn silk	44	13	33		No cage	72[a]
Honey bee pollen substitute mixture	6	No cage	6		0	9
Artificial diet for European corn borers	20	58	4		2	29
Modified artificial diet for European corn borers	4	1	0		0	18
Avg no. eggs per female by cage style		19			29	
Avg no. eggs per female by population density		18		17		36

[a] Two replications.

The average number of eggs laid per female in cages where insects were held at the highest density is much greater than for the other two population levels. However, at the middle density, the females in the large cage laid more eggs per female than did those in the small cage. The averages here are misleading and should not be interpreted as indicating that insects held at the high population density will lay more eggs. Within the range of densities tested there does not seem to be sufficient information to conclude that population density affects fecundity.

Beetles fed on green corn silk, artificial diet for ECB, and cucurbit cotyledons laid the most eggs. Although corn silk is probably the best of the diets tested, the other two appear to be adequate.

While no records were kept on response of the beetles to a diet they seemed to consistently feed very readily on some diets and less so on others. Honey bee pollen substitute mixture seemed to be more palatable than any of the other diets tested. The beetles fed readily on cucurbit cotyledons and seemed to prefer the succulent underside; however, little feeding took place when cucurbit leaves were offered in place of the cotyledons. They fed readily on green corn silk, but did not feed well on the artificial diet for ECB modified with a water extract of corn silk. Perhaps whatever stimulated the beetles to feed on the green corn silk was lacking or in insufficient quantity in the extract. The beetles did

not seem to be either strongly attracted or repelled by the artificial diet for ECB.

Egg handling methods had been very inadequate and very few of the eggs hatched following refrigeration and incubation. For that reason the hatching data cannot be accurately evaluated and are not included in this paper.

The same tests were conducted using NCR. The rates of mortality were very rapid partly due to Beauveria bassiana (Balsamo) Vuillemin, a fungus identified from diseased adults by Dr. Earle S. Raun[2]. The results are not included since the mortality curves would not reflect the effects of diet, caging, and population density on longevity and fecundity. The NCR do not appear to live and reproduce as well as the WCR under the same cage conditions.

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[2] Insect pathologist, USDA, ARS, European Corn Borer Research Laboratory, Ankeny, Iowa.

PART II

HYBRIDIZATION EXPERIMENT

Introduction

The idea was advanced earlier that perhaps D. virgifera and D. longicornis are not biologically isolated species. The geographical range of zones now inhabited by the NCR and WCR overlap considerably, and members of the two species have been seen in the mating position in the field. All stages of the life cycle of WCR and NCR are grossly similar, and the major differences between them seem to be in size, color, and feeding habits of the adult (Peters, 1963). Therefore, hybridization seems likely in these two species.

Hybridization is not rare among insects. It has been reported frequently in the genus Drosophila. Patterson and Stone (1952) in their review of hybridization in this genus reported that 101 cases were discovered in the 32 years following the description of the first hybrid in 1920. Sturtevant (1920) reported that the hybrids produced from a Drosophila melanogaster by Drosophila simulans cross appeared intermediate in all respects in which the parents differed. Hybrids of this cross have also been reported to occur in nature (Mourad and Mallah, 1959). However, D. simulans and D. melanogaster are considered to be reproductively isolated because only one sex in the hybrid is viable and it is sterile.

Hybrids have also been reported to occur in Coleoptera. Trogoderma afrum Priesner and Trogoderma granarium Everts produced fertile hybrids for six generations; as a result they were considered different forms of the same species (Khalifa and Badawy, 1960).

Burkholder (1956) observed members of D. longicornis and D. virgifera in mating position in the field and caged these mating pairs in the laboratory. He collected eggs from two cages of WCR ♀ X NCR ♂ cross but they did not hatch. Eggs were never obtained from the NCR ♀ X WCR ♂ cross. Rearing methods and egg handling techniques have been greatly improved since that time and more meaningful results could be expected. Therefore, both intraspecific and interspecific matings of WCR and NCR were used for this experiment. The tests were first conducted in 1962 and repeated in 1963.

1962 Experiment

Procedures. WCR beetles used in this experiment were collected as pupae at Canton, S. D. and held in the laboratory for emergence. Male and female beetles were held separately. NCR beetles were collected near Brookings, S. D. from a field cage containing 21 corn plants. The adults were collected at intervals of two and one-half hours. The first collection was discarded because virgin females were needed for the mating tests.

All insects were held in the 2.5 in.^{3} polystyrene cages previously described (Fig. 2) and fed on Early Prolific Straightneck squash cotyledons. One pair of beetles was placed in each cage. There were 27 cages of WCR ♀ X WCR ♂, 25 cages of NCR ♀ X NCR ♂, 11 cages of WCR ♀ X NCR ♂, and 25 cages of NCR ♀ X WCR ♂. Eggs obtained from the four matings were handled as described in Procedures for Part I.

Results and conclusions. During this preliminary experiment techniques were lacking and in the process of development. The small pieces of cotton holding eggs dehydrated rapidly although they had been moistened before storing them and at irregular intervals during storage. Later work conducted at the Northern Grain Insects Research Laboratory indicated that the percentage hatch is greatly reduced when the eggs are allowed to dehydrate. All eggs failed to hatch except for a few from the NCR ♀ X NCR ♂ matings. This hatching failure was attributed to inadequate techniques because eggs collected for other experiments from both WCR ♀ X WCR ♂ and NCR ♀ X NCR ♂ matings had hatched under laboratory conditions.

After this experiment had been conducted newly-emerged NCR beetles were observed copulating in the soil. Therefore, the NCR females which had been used in the tests may or may not have mated prior to collection.

Although little data were gathered, the experience gained was used to redesign the experiment.

1963 Experiment

Procedures. In order to insure a supply of virgin NCR and WCR females for the experiment, pupae were field collected and placed in separate containers according to sex. The adults were examined by the method of Smith and Allen (1931) as they emerged to insure the correctness of the initial separation. Beetles were paired and placed in the 25 $in.^3$ cages previously described (Fig. 1). There were 11 cages or replications of WCR♀ X WCR♂, 10 cages of NCR♀ X NCR♂, 28 cages of WCR♀ X NCR♂, and 25 cages of NCR♀ X WCR♂. The beetles were fed on green corn silk since beetles fed on this food in Part I laid more eggs per female than when fed on any other diet.

Only those adults that seemed representative of their species were used in the intraspecific and interspecific matings. Two groups of field collected adults with striking color variance were separated out and placed in separate cages to mate. WCR with bright green pigmentation and NCR with a gray elytron or elytra were caged separately.

All cages were placed on racks (Fig. 3) as described in Part I and held at room temperature. Banks of Westinghouse VHO fluorescent lamps provided a 14-hour photoperiod.

The cellucotton oviposition pads beneath the cages were moistened and checked daily for eggs. The number of eggs and the date they were laid were recorded. Egg papers with the appropriate

identification marked on pieces of plastic plant stakes[3/] were placed on a pad of moist cellucotton, 9 X 5 in., and put into a polyethylene bag (Fig. 7). Studies conducted at the Northern Grain Insects Research Laboratory in 1962 indicated that moisture could be maintained by placing egg papers in a polyethylene bag.

Each bag of eggs was held in the dark at room temperature for two weeks prior to refrigeration at 6°C for 80 days. The two weeks at room temperature allowed for any prediapause embryonic development that might normally occur. The 80-day cold period served to break the egg diapause (George, 1963). At the end of refrigeration time the bags were removed and the individual egg papers placed in polystyrene containers 2 in. in diameter and 7/8 in. deep. A piece of polyethylene was placed between the container and lid to aid in retention of moisture, and the identifying plastic plant stake was taped to the lid. The containers of eggs were placed on galvanized tin trays (Fig. 8) and incubated at 30°C. The temperature was dropped to 25°C when a number of developing embryos failed to hatch. The remaining egg papers were held at this temperature until hatching was apparently complete.

F_1 generation larvae were fed on corn seedlings germinated in dishes of agar (Bigger and March, 1943). The dishes were held at 25°C. Larvae were placed on fresh dishes of corn when the food started to decompose or there was an insufficient supply available.

[3/] Thriftee plant stakes. Lifetime Markers. P. O. Box 216, Clyde, Michigan.

Fig. 7. Small egg papers on cellucotton pad with polyethylene bag.

Fig. 8. Egg incubation boxes on a tray.

Prepupae were removed from the agar plates and placed on barely moist sand in polystyrene containers 2 in. in diameter and 7/8 in. deep (Hintz and George, 1964). Following pupation, sex of the pupa was determined and the container labeled appropriately. The adults were allowed to emerge in these containers.

Results and conclusions. Data recorded on the interspecific and intraspecific matings are summarized in Table 4. Eggs were produced by all four P_1 mating combinations, although some replications did not produce any eggs (Table 4, columns 1 and 2). More of the replications involving a WCR female produced eggs than replications involving a NCR female (Table 4, column 2); the two matings involving WCR females produced more eggs than those involving NCR females (Table 4, column 5). The average number of eggs laid per female was greater for matings involving WCR females than for NCR females (Table 4, column 7). The figures for average number of eggs produced per female were similar whether computed using all females or only egg-laying females (Table 4, columns 7 and 8).

The eggs were incubated at 30°C until 6 December 1963. Some of the eggs produced by nine females of the two interspecific crosses exhibited either malformed embryos or apparently normal embryos which did not hatch, or both. Since it is unlikely that rootworm eggs in the field ever experience 30°C soil temperatures, the temperature in the incubator was dropped to 25°C. Following the reduction in temperature no visibly malformed embryos developed and larvae

Table 4. Summary of intraspecific and interspecific matings of D. virgifera (WCR) and D. longicornis (NCR). 1963.

P_1 matings	Replications[a]	P_1 ♀ producing eggs	P_1 ♀ producing F_1 larvae	Extreme no. of eggs produced per P_1	Total eggs produced	Eggs produced[b]
	(1)	(2)	(3)	(4)	(5)	(6)
WCR♀ X WCR♂	11	11	7	4-748	3215	2496
NCR♀ X NCR♂	10	6	3	0-136	284	252
WCR♀ X NCR♂	28	26	10	0-602	6318	3329
NCR♀ X WCR♂	25	19	3	0-341	1297	197

P_1 matings	Avg eggs produced per P_1 ♀ [c]	Avg eggs produced per P_1 ♀ [d]	Total eggs hatched	% of all eggs hatched	% of eggs hatched[e]
	(7)	(8)	(9)	(10)	(11)
WCR♀ X WCR♂	292	292	1507	47	60
NCR♀ X NCR♂	28	47	12	4	5
WCR♀ X NCR♂	226	243	75	1	2
NCR♀ X WCR♂	52	68	137	11	70

[a] One pair per cage.
[b] Including only those pairs that produced F_1 larvae.
[c] Including all P_1 ♀.
[d] Including only those P_1 ♀ which produced eggs.
[e] Including only the eggs produced by those P_1 matings that produced F_1 larvae.

hatched from eggs produced by those same females. The deformed larvae were probably the result of high temperatures rather than genetic characteristics.

Larvae were produced from each of the four combinations (Table 4, column 3), although only 37% of the ovipositing females produced viable eggs. A much higher percentage egg hatch was obtained from matings including a WCR male than from matings involving a NCR male (Table 4, columns 10 and 11). It appears that while WCR females may be more fecund than the NCR females, the level of fertility of the resulting eggs is determined by the male involved in the mating.

Mortality of the F_1 larvae and pupae was not recorded. The rate of mortality was high due to disease, and any record of the number of insects completing the life cycle would not reflect the viability and vigor of the matings. Dr. Earle S. Raun[4/] examined some of the diseased specimens and attributed death to a granulosis virus with secondary bacterial infections.

Larvae from three of the four mating combinations were reared through their life cycle. Three F_1 adults each were produced from the NCR ♀ X WCR ♂ and WCR ♀ X NCR ♂ crosses. Many of the larvae produced from the WCR ♀ X WCR ♂ parent matings were used for other experiments in the laboratory and only five F_1 adults were

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[4/] Insect pathologist, USDA, ARS, European Corn Borer Research Laboratory, Ankeny, Iowa.

reared. Twelve larvae hatched from eggs produced by the NCR♀ X NCR♂ parent matings, but they were used for other experimental purposes and none of them reached adulthood.

The adults produced from the three matings all appeared morphologically similar to D. virgifera. The color pattern of the WCR seems to be dominant in the F_1 whether contributed by either the male or the female. Some adults from all matings developed color variations several weeks after emergence. This variation appeared to be like that observed in the field populations. Duplication of this color variance in the laboratory indicates that perhaps field populations of WCR and NCR are mating as suspected.

Females in the two separate cages holding WCR with bright green pigment and NCR with a gray elytron both produced F_1 adults. The offspring from bright green pigmented WCR beetles appeared to be as varied in color as those normally found in a field population. The one F_1 adult produced from the caged NCR appeared grossly identical to a WCR. Since these beetles were field collected there is no way to determine if they had mated prior to collection. If the NCR females had not mated with WCR males then the genes for the WCR color pattern can be carried without complete expression. If they had mated with WCR males before the time of collection then the production of a WCR type offspring from a NCR female indicates hybridization is occurring in the field. This may be a significant factor in vigor, rapid build-up of WCR populations, and decline of the NCR populations.

No cytological examinations were incorporated into this study. However, a cytogeneticist[5/] examined a F_1 male of *D. longicornis* ♀ X *D. virgifera* ♂ parentage. He stated that from the limited material available there was no cytological evidence of the individual being a hybrid between two biologically valid species. The identification of inherited traits and the mechanism of their transmission awaits a more complete genetic study.

5/ Dr. S. G. Smith, Head, Section of Cytology and Genetics, Forest Insect Laboratory, Sault Ste. Marie, Ontario, Canada.

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APPENDIX

Table 5. Artificial diet for European corn borers, Ostrinia nubilalis.[a]

	Amount used	Dry diet
	g	%
Carrier:		
Distilled water	255.00[b]	
Bacto-Agar	6.60	12.6
Glucose	10.50	20.0
Casein, vitamin-free[c]	10.50	20.0
Cholesterol	0.85	1.6
Corn oil containing 1% alpha tocopherol	0.50	1.0
Salts mixture No. 2, U.S.P. XIII[c]	1.30	2.5
Choline chloride	0.12	0.2
Brewers yeast U.S.P.	6.90	13.1
Leaf factor	13.80	26.3
Mold Inhibitor Mixture:[d]		
n-butyl p-hydroxybenzoate	0.60	1.1
Sorbic acid	0.60	1.1

[a] Becton, A. J., B. W. George, and T. A. Brindley. 1962. Continuous rearing of European corn borer larvae on artificial medium. p. 165. Iowa State J. Science 37(2).

[b] Plus 10% to compensate for evaporation during cooling.

[c] Nutritional Biochemicals, Inc., Cleveland, Ohio.

[d] Added in 95% ethyl alcohol.